Chemopreventive Effects of 24R,25-Dihydroxyvitamin D₃, a Vitamin D₃ Derivative, on Glandular Stomach Carcinogenesis Induced in Rats by N-Methyl-N′-nitro-N′-nitrosoguanidine and Sodium Chloride

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ABSTRACT

The modifying effects of 24R,25-dihydroxyvitamin D₃ [24R,25(OH)₂D₃], a vitamin D₃ derivative, on glandular stomach carcinogenesis were investigated in male Wistar rats by N-methyl-N'-nitro-N'-nitrosoguanidine (MNNG) and sodium chloride exposure during the postinitiation phase. A total of 130 male 6-week-old rats was divided into five groups. Groups 1–3 (consisting of 30 rats/group) were given MNNG in drinking water at a concentration of 100 ppm and were simultaneously fed a diet supplemented with 10% NaCl for 8 weeks. They were fed a diet containing either 5.0 ppm (group 1) or 2.5 ppm (group 2) 24R,25(OH)₂D₃ or were kept on the basal diet alone (group 3) for the following 57 weeks. Rats in groups 4 and 5 were given 24R,25(OH)₂D₃ as sodium chloride exposure during the postinitiation phase. A total of 130 male Wistar rats by Af methyl-JV'-nitro-A'-nitrosoguanidine (MNNG) and sodium chloride exposure during the postinitiation phase. A total of 130 male 6-week-old rats was divided into five groups. Groups 1–3 (consisting of 30 rats/group) were given MNNG in drinking water at a concentration of 100 ppm and were simultaneously fed a diet supplemented with 10% NaCl for 8 weeks. They were fed a diet containing either 5.0 ppm (group 1) or 2.5 ppm (group 2) 24R,25(OH)₂D₃ or were kept on the basal diet alone (group 3) for the following 57 weeks. Rats in groups 4 and 5 were given 24R,25(OH)₂D₃ as

INTRODUCTION

Epidemiological studies have suggested that elevated calcium intake and dietary or endogenously synthesized vitamin D may decrease the risk of gastrointestinal cancer (1). In fact, calcium supplementation has been shown to inhibit colon carcinogenesis in rats (2, 3). We have also shown that calcium chloride injection has a dose-dependent chemopreventive effect on glandular stomach carcinogenesis in rats when given during the postinitiation phase (4). In addition, 1,25(OH)₂D₃, a major active form of vitamin D, has been proven to inhibit colon (5, skin (6, 7), and mammary (8) carcinogenesis in rats or mice, although this vitamin D derivative has been reported to cause excessive hypercalcemia (5, 9). Another active form of vitamin D, 24R,25(OH)₂D₃, has been reported to cause milder hypercalcemia than 1,25(OH)₂D₃ (9, 10). It is known that 24R,25(OH)₂D₃ induces some histopathological as well as pharmacological changes in bone tissue (11–14), but no information on the action of cancer prevention with this chemical has yet been available.

MATERIALS AND METHODS

Chemicals and Animals. MNNG was a commercially available preparation obtained from Aldrich Chemical Co. (Milwaukee, WI). NaCl purity >99.5% was purchased from Wako Pure Chemicals, Inc. (Osaka, Japan). 24R,25(OH)₂D₃ was generously donated by Kuureha Chemical Co. Ltd. (Tokyo, Japan). Male 6-week-old Wistar rats (Japan SLC Inc., Shizuoka, Japan) were housed five animals per polycarbonate cage and were maintained under standard laboratory conditions (room temperature, 23 ± 2°C; relative humidity, 60 ± 5%; a 12 h/12 h light/dark cycle). They were fed a basal diet, Oriental MF (Oriental Yeast Co. Ltd., Tokyo, Japan), supplemented with or without the test chemical.

Experimental Protocol. As shown in Fig. 1, rats in groups 1–3 (each group consisted of 30 animals) were given MNNG in drinking water at a concentration of 100 ppm and were simultaneously fed a diet supplemented with 10% NaCl for 8 weeks. They were then given a diet supplemented with 24R,25(OH)₂D₃ at doses of 5.0 ppm (group 1), 2.5 ppm (group 2), or 0 ppm (group 3) for the following 57 weeks. The doses of 24R,25(OH)₂D₃ were chosen based on the experiment in rats (12). The 20 rats of group 4 were fed 5.0 ppm 24R,25(OH)₂D₃ alone during the 57 weeks (similar to group 1) but did not receive the MNNG + NaCl treatment. Group 5 (20 animals) served as a nontreatment control. The animals were observed daily for toxicological symptoms and were weighed once a month. At the end of experiment (65 weeks), all surviving animals were killed and autopsied. At autopsy, lung, liver, kidney, and heart were excised and weighed. The stomachs were subjected to a particularly careful macroscopic examination before fixation in 10% buffered formalin and then were cut into longitudinal strips (3-mm wide) for examination of the entire gastric mucosa. After processing for histology by routine methods, sections were stained with H&E. Calcium and phosphorus levels in urine samples collected over 24-h periods at weeks 10, 30, and 62 were analyzed using a Hitachi 736–60E autoanalyzer (Hitachi Ltd., Tokyo, Japan). Serum biochemical parameters were also determined (using a Hitachi 736–60E autoanalyzer) in blood collected from the abdominal aorta immediately before autopsy.

Statistical Analysis. The data for lesion incidences were analyzed using Fisher's exact test, and the data for lesion multiplicities, organ weights, and biochemical quantitation were examined using Student's t test.

RESULTS

Consumption of 24R,25(OH)₂D₃ and Mortality. The mean daily intake of 24R,25(OH)₂D₃ in groups 1–5 (calculated from food consumption and mean body weight values) was 25.5, 13.1, 0, 23.2, and 0 μg/100 g body weight, respectively. The intake of 24R,25(OH)₂D₃ correlated well with the dose levels used in the present study. One animal in each of groups 1–3 was found dead at weeks 60, 52, and 63,
respectively. All surviving rats were included in the effective numbers.

**Body and Organ Weights.** Although body weight gain was remarkably suppressed in animals given the MNNG + NaCl treatment as compared to rats without the treatment, especially in the beginning of the experiment, the body weights had recovered by the termination of the experiment (Fig. 2). Regardless of administration of 24R,25(OH)2D3, rats in the groups exposed to MNNG + NaCl (groups 1–3) showed body weight curves similar to those of the groups without exposure (groups 4 and 5) toward termination of the experiment, indicating that even the high dose of 24R,25(OH)2D3 did not affect body weight gain. In group 1, the absolute heart weights were significantly depressed (P < 0.01), and the relative kidney weights were significantly elevated (P < 0.01) as compared to the respective values of the control (group 3). There were no statistical differences among groups in the lung and liver weights, with or without the MNNG + NaCl or the 24R,25(OH)2D3 treatment.

**Effects of 24R,25(OH)2D3 on Glandular Stomach Carcinogenesis.** Cancerous and precancerous lesions in the glandular stomach were all diagnosed as adenocarcinomas and atypical hyperplasias (Fig. 3), respectively, as described previously (4, 16, 17). As shown in Table 1, the incidences of atypical hyperplasias of glandular stomachs in groups 1 (21%) and 2 (38%) were significantly lower (P < 0.01 and P < 0.05) than in group 3 (66%). The total incidence of combined atypical hyperplasias and adenocarcinomas in group 1 (24%) was also significantly lower (P < 0.01) than that of group 3 (70%), although the development of adenocarcinomas was not frequent in MNNG-treated rats, regardless of 24R,25(OH)2D3 treatment. The total incidence of combined proliferative lesions was dose-dependently decreased by 24R,25(OH)2D3 treatment in both fundic and pyloric mucosa and was statistically significant in the former case (P < 0.01). No rats in groups 4 and 5 developed adenocarcinomas or atypical hyperplasias. As shown in Table 2, the mean numbers of atypical hyperplasias plus adenocarcinomas per animal, i.e., multiplicities, in the glandular stomachs of groups 1 (0.31) and 2 (0.66) were significantly decreased (P < 0.01 and P < 0.05, respectively) as compared to the group 3 value (1.21). The multiplicities of atypical hyperplasias in groups 1 (0.24) and 2 (0.59) were also significantly smaller (P < 0.01 and P < 0.05, respectively) than that of group 3 (1.14). Accordingly, the treatments of 24R,25(OH)2D3 significantly reduced the development of stomach neoplasia and preneoplasia in a dose-dependent manner.

**Urinary Excretion of Calcium and Phosphorus and Serum Biochemistry.** Urinary calcium levels were significantly increased by the administration of 24R,25(OH)2D3 at all three time points, regardless of the MNNG + NaCl treatments (Fig. 4). The calcium level was highest at week 30 in all groups. No apparent changes in the urinary phosphorus levels were detected, although dose-independent decreases or increases were infrequently noted (data not shown). Serum phosphorus levels were comparable among the groups, with the exception of the group 4 value, which was significantly higher (P < 0.05) than the control value. The levels of blood urea nitrogen were slightly but significantly higher (P < 0.05) in group 1 than in group 3. There were no significant changes in the other serum parameters including calcium (data not shown).

**Histopathology of the Other Organs.** No apparent toxic damage was recognized histopathologically in the liver, kidney, lung, and heart.

**DISCUSSION**

The results of the present study clearly indicate that 24R,25(OH)2D3 has a chemopreventive action against glandular stomach carcinogenesis by MNNG and NaCl in rats. Because atypical hyperplasias are generally accepted as preneoplastic lesions (4, 17) and the inhibitory effect of this agent was confirmed in the early lesions toward stomach cancers, additional studies regarding: (a) comparison with the other active metabolites of vitamin D like 1,25(OH)2D3; (b) reversibility of the observed inhibitory effect; and (c) the effects during the initiation phase could warrant our results. It has been well documented that vitamin D is a promising chemopreventive agent against colon carcinogenesis (1, 2). The underlying mechanisms remain to be elucidated, but several hypotheses have been proposed. For example, in addition to an increase in calcium intake (2, 18), other physiological activities of active vitamin D, such as regulation of terminal cell differentiation (19, 20), influence on immune systems (21, 22), and inhibition of ornithine decarboxylase induction (23, 24), may contribute to the chemopreventive action. Furthermore, it has

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**Table 1. The Incidences of Atypical Hyperplasias of Glandular Stomach**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Diet</th>
<th>5.0 ppm 24R,25(OH)2D3</th>
<th>2.5 ppm 24R,25(OH)2D3</th>
<th>Basal Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>MNNG</td>
<td>100 ppm MNNG in drinking water + 10% NaCl in diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>MNNG</td>
<td></td>
<td>2.5 ppm 24R,25(OH)2D3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>Basal Diet</td>
<td></td>
<td></td>
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</tbody>
</table>
also been demonstrated that ornithine decarboxylase induction in the glandular stomach of rats by NaCl, a stomach tumor promoter, is inhibited by 1(OH)D3 (24). Oral preadministration of 1(OH)D3 provides a nonphysiological amount of 1,25(OH)2D3 because 25-hydroxylation of 1(OH)D3 in the liver is not regulated by serum calcium levels (24, 25). Therefore, it is suggested that 1,25(OH)2D3 may have an antipromoting effect against gastric carcinogenesis as well as carcinogenesis in skin and colon (24). Nevertheless, dietary application of 1,25(OH)2D3 has been limited, partly because of the disadvantage of an excessive increase in serum calcium levels (5, 9).

Notwithstanding the presence of information for the influence on bone cartilage (11-14), the physiological effects of 24R,25(OH)2D3, a metabolite of 25(OH)D3 in the kidney, are not well known. It has been shown that the ratios of vitamin D3 metabolites in the kidney depend on the serum calcium levels. Namely, 1,25(OH)2D3 is predominantly produced when serum calcium levels are lower than 9 mg/dl, whereas production of 24R,25(OH)2D3 is increased when serum calcium levels are higher than this value (26). In relation to such data, 24R,25(OH)2D3 is known to induce little, if any, hypercalcemia (9, 10). In fact, 24R,25(OH)2D3 did not induce hypercalcemia at any dose in the present study, although the chemical generated calcemia at any dose in the present study, although the chemical generated

![Graph of Urinary Excretion of Calcium](image)

**Fig. 4.** Urinary excretion of calcium in rats receiving MNNG + NaCl and/or 24R,25(OH)2D3 treatment at weeks 10 (C), 30 (E), and 62 (S). Values represent the mean; bars, SD. *Significantly different from group 3 at P < 0.01. # and ##, significantly different from group 5 at P < 0.05 and P < 0.01, respectively.

**REFERENCES**


VITAMIN D PREVENTS STOMACH CARCINOGENESIS


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